

Stability test program:

% 1->phlf

% 2->lac

% 3->tet

%define the maximum production rate of repressors

x1 = 1;

x2 = 1;

x3 = 1;

%define the dissociation constant of repressors to promoters

K1 = 1260;

K2 = 8*10⁽⁻⁷⁾;

K3 = 1*10⁽⁻⁸⁾;

%define the degradation constant of repressors

k1 = 0.01158;

k2 = 0.12;

k3 = 0.1386;

%define hill coefficient of repressors to promoters

n1 = 2.16;

n2 = 2;

n3 = 2;

syms y1 y2 y3;

y=[y1,y2,y3];

f=[x2/(1+(y2/K2)ⁿ²)+x3/(1+(y3/K3)ⁿ³)-k1*y1, x1/(1+(y1/K1)ⁿ¹)+x3/(1+(y3/K3)ⁿ³)-
k2*y2,x1/(1+(y1/K1)ⁿ¹)+x2/(1+(y2/K2)ⁿ²)-k3*y3];

[ye1,ye2,ye3]=solve(f(1),f(2),f(3));

J=jacobian(f,y);

```
for k=1:length(ye1)
    y1=eval(ye1(k));
    y2=eval(ye2(k));
    y3=eval(ye3(k));
    A=subs(J);
    lambda=eig(A);
    disp(lambda);
end
```

Prediction Model Program:

```
function tristable_trial_v3_parameters
clear all
clc

%find ODE path (do not edit)
odefunction=@computeprotein;

%define rate of transcription
maxtx_rate_mrna1 = 1;
maxtx_rate_mrna2 = 1;
maxtx_rate_mrna3 = 1;

%define rate of mRNA degradation = ln2/half-life
kdeg_mrna1 = 0.12;
kdeg_mrna2 = 0.12;
kdeg_mrna3 = 0.12;

%define rate of translation from each mRNA
maxtl_rate1 = 1;
maxtl_rate2 = 1;
maxtl_rate3 = 1;
```

%define rate of protein degradation

kdeg_protein_a = 0.12;

kdeg_protein_b = 0.12;

kdeg_protein_c = 0.12;

kdeg_protein_o1 = 0.12;

kdeg_protein_o2 = 0.12;

kdeg_protein_o3 = 0.12;

%define Kd (dissocation constant) for [DNA-Repressor] interaction

kd1_1 = 1e-8;

kd1_2 = 1e-8;

kd1_3 = 1e-8;

%define hill coefficient (n)

hill1_1 = 2;

hill1_2 = 2;

hill1_3 = 2;

hill2_1 = 2;

hill2_2 = 2;

hill2_3 = 2;

%define Kd (dissocation constant) for [Repressor-inducer] interaction

kd2_1 = 1.5e-8;

kd2_2 = 1.5e-8;

kd2_3 = 1.5e-8;

%set initial mRNA concentration

species0(1)=0;

species0(2)=0;

species0(3)=0;

%set initital protein concentration

species0(4)=0;

```

species0(5)=0;
species0(6)=0;
species0(7)=0;
species0(8)=0;
species0(9)=0;
% transpose matrix (do not edit)
species0 = species0';

%% define modelling duration, time pulse and pulse strength

%set total time to be modelled
t_length = 1000;

[t,totalspeciesx] = ode45(odefunction, [0,t_length], species0); %simulation (do not edit)
%%

%plot the figure
x1 = totalspeciesx(:,1);
x2 = totalspeciesx(:,2);
x3 = totalspeciesx(:,3);
y1 = totalspeciesx(:,4);
y2 = totalspeciesx(:,5);
y3 = totalspeciesx(:,6);
o1 = totalspeciesx(:,7);
o2 = totalspeciesx(:,8);
o3 = totalspeciesx(:,9);

inducer1 = 0*(t>=300&t<=550);
inducer2 = 1e-3*(t>=100&t<=200)+0*(t>=1800&t<=3500);
inducer3 = 0*(t>=1500&t<=1600);

fig = figure(1);

```

```
set(fig, 'Name', 'Time Plot: Tri-state');

subplot(4,3,1)
plot(t,inducer1,'m');
title('Inducer 1 Concentration Over Time');
xlabel('time (min)');
ylabel('concentration (M)');
axis([0,t_length,0,1.5e-3]);
legend('inducer 1');

subplot(4,3,2)
plot(t,inducer2,'m');
title('Inducer 2 Concentration Over Time');
xlabel('time (min)');
ylabel('concentration (M)');
axis([0,t_length,0,1.5e-3]);
legend('inducer 2');

subplot(4,3,3)
plot(t,inducer3,'m');
title('Inducer 3 Concentration Over Time');
xlabel('time (min)');
ylabel('concentration (M)');
axis([0,t_length,0,1.5e-3]);
legend('inducer 3');

subplot(4,3,4)
plot(t,x1,'r');
title('mRNA 1 Concentration Over Time');
xlabel('time (min)');
ylabel('concentration (per cell)');
axis([0,t_length,0,25]);
```

```
legend('mRNA 1');
```

```
subplot(4,3,5)
```

```
plot(t,x2,'r');
```

```
title('mRNA 2 Concentration Over Time');
```

```
xlabel('time (min)');
```

```
ylabel('concentration (per cell)');
```

```
axis([0,t_length,0,25]);
```

```
legend('mRNA 2');
```

```
subplot(4,3,6)
```

```
plot(t,x3,'r');
```

```
title('mRNA 3 Concentration Over Time');
```

```
xlabel('time (min)');
```

```
ylabel('concentration (per cell)');
```

```
axis([0,t_length,0,25]);
```

```
legend('mRNA 3');
```

```
subplot(4,3,7);
```

```
plot(t,y1,'b')
```

```
title('Repressor 1 Concentration Over Time');
```

```
xlabel('time (min)');
```

```
ylabel('concentration (per cell)');
```

```
axis([0,t_length,0,450]);
```

```
legend('repressor 1');
```

```
subplot(4,3,8);
```

```
plot(t,y2,'b')
```

```
title('Repressor 2 Concentration Over Time');
```

```
xlabel('time (min)');
```

```
ylabel('concentration (per cell)');
```

```
axis([0,t_length,0,450]);
```

```
legend('repressor 2');

subplot(4,3,9);
plot(t,y3,'b')
title('Repressor 3 Concentration Over Time');
xlabel('time (min)');
ylabel('concentration (per cell)');
axis([0,t_length,0,450]);
legend('repressor 3');
```

```
subplot(4,3,10);
plot(t,o1,'b')
title('Output 1 Concentration Over Time');
xlabel('time (min)');
ylabel('concentration (per cell)');
axis([0,t_length,0,450]);
legend('output 1');
```

```
subplot(4,3,11);
plot(t,o2,'b')
title('Output 2 Concentration Over Time');
xlabel('time (min)');
ylabel('concentration (per cell)');
axis([0,t_length,0,450]);
legend('output 2');
```

```
subplot(4,3,12);
plot(t,o3,'b')
title('Output 3 Concentration Over Time');
xlabel('time (min)');
ylabel('concentration (per cell)');
axis([0,t_length,0,450]);
```

```
legend('output 3');
```

```
%% Equation to be modelled
```

```
function dspecies = computeprotein(t,species)
```

```
% define pulse
```

```
inducer1 = 0*(t>=300&t<=550);
```

```
inducer2 = 1e-3*(t>=100&t<=200)+0*(t>=1800&t<=3500);
```

```
inducer3 = 0*(t>=1500&t<=1600);
```

```
inducer1_term = 1/(1+(inducer1/kd2_1)^hill2_1);
```

```
inducer2_term = 1/(1+(inducer2/kd2_2)^hill2_2);
```

```
inducer3_term = 1/(1+(inducer3/kd2_3)^hill2_3);
```

```
% describes mRNA production and degradation
```

```
dspecies(1) = maxtx_rate_mrna1/(1+(species(4)*inducer1_term/kd1_1)^hill1_1) -  
kdeg_mrna1*species(1);
```

```
dspecies(2) = maxtx_rate_mrna2/(1+(species(5)*inducer2_term/kd1_2)^hill1_2) -  
kdeg_mrna2*species(2);
```

```
dspecies(3) = maxtx_rate_mrna3/(1+(species(6)*inducer3_term/kd1_3)^hill1_3) -  
kdeg_mrna3*species(3);
```

```
% describes protein production and degradation
```

```
dspecies(4) = maxtl_rate2*species(2) + maxtl_rate3*species(3) - kdeg_protein_a*species(4);
```

```
dspecies(5) = maxtl_rate1*species(1) + maxtl_rate3*species(3) - kdeg_protein_b*species(5);
```

```
dspecies(6) = maxtl_rate2*species(2) + maxtl_rate1*species(1) - kdeg_protein_c*species(6);
```

```
dspecies(7) = maxtl_rate1*species(1) - kdeg_protein_o1*species(7);
```

```
dspecies(8) = maxtl_rate2*species(2) - kdeg_protein_o2*species(8);
```

```
dspecies(9) = maxtl_rate3*species(3) - kdeg_protein_o3*species(9);
```

```
dspecies = dspecies'; %transpose matrix (do not edit)
```


end

end